approximated by Hooke's law, it is not clear if that is still valid for large bending angles. The experimental evidence is controversial. We explore the strong bending regime of the double helix using a model that represents the solvent implicitly, which allows for greater efficiency. First, we are able to reproduce results of Strauss and Maher. Next, we compare the energetics of weakly and strongly bent DNA. We find that Hooke's law is violated for strongly bent DNA and discuss the energetic contribution that may be responsible for the effect.

87-Plat

Mechanisms for Efficient TRNA Translocation through the Ribosome

Lars V. Bock[1], Christian Blau[1], Gunnar F. Schröder[2], Niels Fischer[1], Holger Stark[1], Marina V. Rodnina[1], Andrea C. Vaiana[1], Helmut Grubmüller[1].
[1]MPI for Biophysical Chemistry, Göttingen, Germany, [2]Forschungszentrum Jülich, Jülich, Germany.

After peptide bond formation the transfer RNAs (tRNAs) bound to the ribosome translocate by more than 7 nm to adjacent binding sites, accompanied by large-scale conformational motions of the ribosome. Combining cryo-EM reconstructions of translocation intermediates (Fischer, Nature 2010) with high resolution crystal structures, we obtained 13 near-atomic resolution structures. The quality of these structures was validated using recent crystal structures and subsequently all-atom molecular dynamics simulations of the fully solvated 70S ribosome were carried out for each of the 13 intermediate states, totaling 1.5 μs. The obtained dynamics within the intermediate states allowed us to estimate transition rates between states for motions of the L1-stalk, tRNAs and intersubunit rotations. These rates revealed rapid motions of the L1-stalk and the small subunit on sub-microsecond timescales, whereas the tRNA motions were seen to be rate-limiting for most transitions. By calculating the free energy of interaction between L1-stalk and tRNA, we obtained molecular forces revealing that the L1-stalk is actively pulling the tRNA from P to E site, thereby overcoming barriers for the tRNA motion. Further, ribosomal proteins L5 and L16 guide the tRNAs by 'sliding' and 'stepping' mechanisms involving key protein-tRNA contacts, explaining how tRNA binding affinity is kept sufficiently constant to allow rapid translocation despite large-scale displacements.

88-Plat

Coarse-Grained Computational Characterization of RNA Nanocube Flexibility Correlates with Experiments

Wojciech K. Kasprzak[1], Kirill A. Afonin[2], Eckart Bindewald[1], Praneet S. Puppala[2], Tae-Jin Kim[2], Michael T. Zimmermann[3], Robert L. Jernigan[3], Bruce A. Shapiro[2].
[1]Basic Science Program, SAIC-Frederick, Inc., Frederick National Laboratory for Cancer Research, Frederick, MD, USA, [2]CCR Nanobiology Program, Frederick National Laboratory for Cancer Research, Frederick, MD, USA, [3]Department of Biochemistry, Biophysics and Molecular Biology, Iowa State University, Ames, IA, USA.

The emerging field of RNA-based nanotechnology can benefit from the development of new computational methods capable of helping in the design and characterization of nano-scale particles, leading to the development of qualitatively new structures and novel therapeutics. We have approached the computer-aided design process by creating a pipeline of tools, starting with a database of n-way junctions and kissing-loops called RNAJunction. This database provides building blocks for our programs, such as NanoTiler and RNA2D3D, which use them to design 3D models of RNA nanostructures. First, the building blocks are treated as rigid objects. Then, just as the natural RNA is shaped (deformed) by the larger structural contexts, our programs allow for deformations to be applied in order to produce fully assembled models. To assess the realistic limits of these deformations, we consider flexibility data available as alternative structures in databases as well as results of Molecular Dynamics (MD) simulations at the atomic resolution level and coarse-grained computational methods. Here we present an example of the modeling process including RNA flexibility information for three nanocube model variants and a novel application of a coarse-grained Anisotropic Network Model (ANM) to the RNA nanostructure characterization. The predictions of different efficiency of assembly for three nanocube variants, based on the exploratory modeling, were confirmed in in vitro experiments. The ANM simulations showed that the dynamics of the full nanostructure has to be considered in order to explain the differences between the size of the initial static models and that of the experimentally measured nanoparticles, thus bringing the computational and the experimental results into agreement. The ANM simulations also offered an additional insight into the assembly yields and the difference in the melting temperatures of the cube variants.

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89-Plat

Conformational Transitions of Nucleic Acids under External Forces: Computer Simulations and a Stochastic Theory for their Kinetics

Ioan Andricioaei.
University of California - Irvine, Irvine, CA, USA.

I will present molecular dynamics simulations of several examples of conformational transitions that nucleic acids and their complexes undergo upon the application of external forces and/or torques:
(1) DNA supercoil relaxation by topoisomerases,
(2) the condensation of DNA by dendrimers and,
(3) RNA unfolding.
Then I will showcase the use of the formalism of stochastic path integrals to deduce the kinetics of these transitions, from simulation trajectories or experimental single molecule recordings of the transition, under other conditions that those that are actually simulated or recorded.

Platform: Cardiac Muscle I

90-Plat

Using FRET to Characterize the Actomyosin Complex in Cardiac Muscle

Lucia Chen[1], Valentina Caorsi[1,2], Christopher Toepfer[1,2], Weihua Song[1,3], Steven B. Marston[1,3], Mike A. Ferenczi[1,2].
[1]Imperial College London, London, United Kingdom, [2]Molecular Medicine, NHLI, London, United Kingdom, [3]Myocardial Function, NHLI, London, United Kingdom.

Elucidating actomyosin interactions within cardiac muscle is key to understanding molecular mechanisms of force generation in the heart. Precise myosin-actin interactions throughout the power stroke are still unclear and study of actomyosin within functional muscle systems is required [1].
We exploit the nanometre precision of Förster resonance energy transfer (FRET) to study the actomyosin complex in healthy and diseased cardiac muscle, using mouse papillary muscle. The distance between the essential light chain (ELC)-AlexaFluor488 (labelled at a single cysteine in position 180 of a modified ELC exchanged into the fibre [2]) and Actin-AlexaFluor594-Phalloidin is evaluated by the acceptor-photobleaching method. The hypertrophic cardiomyopathy-causing actin mutation, E99K [3], was also studied in terms of the ELC-Actin distance, and compared with wild-type results.
The mean FRET efficiencies evaluated for wild-type and E99K relaxed-state fibres were 15.1% and 15.0% respectively ($p>0.05$), corresponding to ELC-Actin distances of 87.6Å and 87.2Å. Rigor-state FRET efficiencies were approximately 10% lower than in the relaxed-state, corresponding to distances around 20Å shorter. Our preliminary results suggest: i) E99K actin-mutation does not affect the acto-myosin structures in terms of FRET efficiencies evaluated; ii) ELC-Actin distance in cardiac fibres is within the FRET range; iii) ELC-Actin distance in relaxed cardiac fibres is shorter than rigor-state distances.
In conclusion, FRET is viable for studying nanometre distances in intact cardiac tissue and provides a new perspective into the study of cardiac contraction. Furthermore, in contrast to skeletal fibres [4], the ELC-Actin distance in rigor cardiac fibres is within the range for FRET, indicating that cardiac and skeletal muscle may possess differing cross-bridge conformations.

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91-Plat

Length Dependence of Force Generation is Controlled by Phosphorylation of cTnI at Serines 23/24

Laurin M. Hanft[1], Brandon J. Biesiadecki[2], Kerry S. McDonald[1].
[1]University of Missouri, Columbia, MO, USA, [2]The Ohio State University, Columbus, OH, USA.

The steepness of the Frank-Starling relationship is modulated by numerous physiological factors including beta-adrenergic stimulation, which steepens the relationship. This arises in part from increased myofibrillar length dependence of force and power by PKA, a downstream signaling molecule of the beta-adrenergic system. Since PKA has multiple myofibrillar substrates including titin, myosin binding protein-C (MyBP-C), and cardiac troponin I (cTnI), we sought to define if phosphorylation of one of these molecules was sufficient to control length-tension relationships. We focused on cTnI since (i) we previously observed a relationship between cTnI phosphorylation and the steepness of ventricular function curves in rat working hearts, (ii) 2D-DIGE indicated a distribution of cTnI phosphorylation states consistent with our previous observation of two populations of length-tension relationships (one shallow the